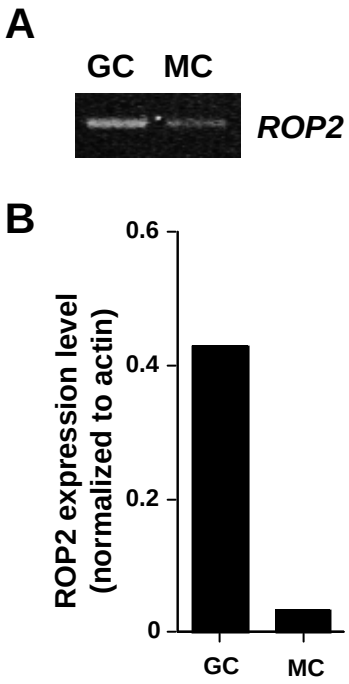
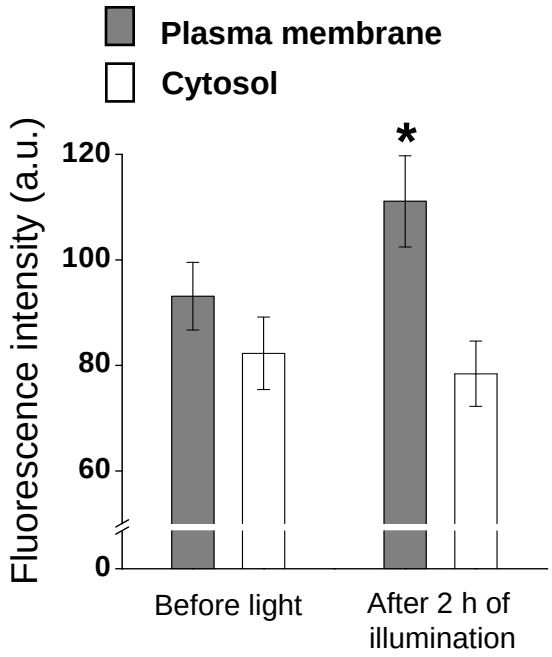
Supplemental Data. Jeon et al. (2007). The *Arabidopsis* Small G Protein ROP2 is activated by light in guard cells and inhibits light-induced stomatal opening.

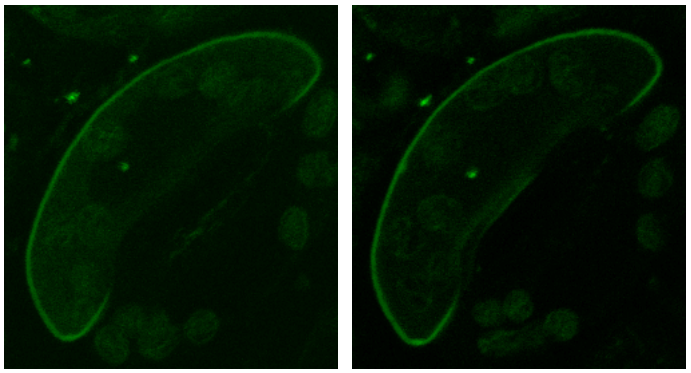


Supplemental Figure 1. *ROP2* gene expression in Arabidopsis guard cells.
(A) RT-PCR result, a technical replicate of Figure 1A.
(B) Microarray result obtained from a single hybridization of ATH1 chips with guard cell and mesophyll cell RNAs pooled from two independent protoplast preparations as described previously (Leonhardt et al., 2004).



Supplemental Figure 2. Fluorescence intensity values of GFP-AtROP2 at the plasma membrane (PM) and in the cytosol before and after illumination with white light at intensities of 250 µmol $m^{-2}s^{-1}$. See Materials and Methods for assay protocol. Note that the fluorescence intensity of GFP-AtROP2 at the PM increased after 2 h of illumination ($P < 0.02$, asterisked), while that of cytosol did not change significantly (mean ± S. E., n=11). a.u.; arbitrary unit.

A



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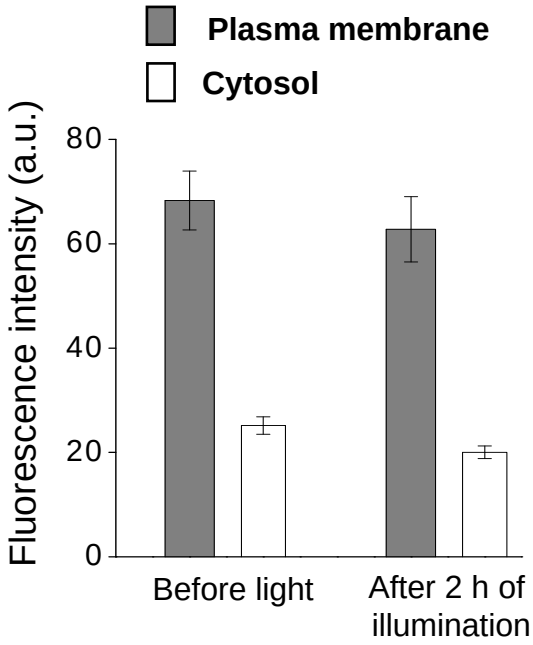
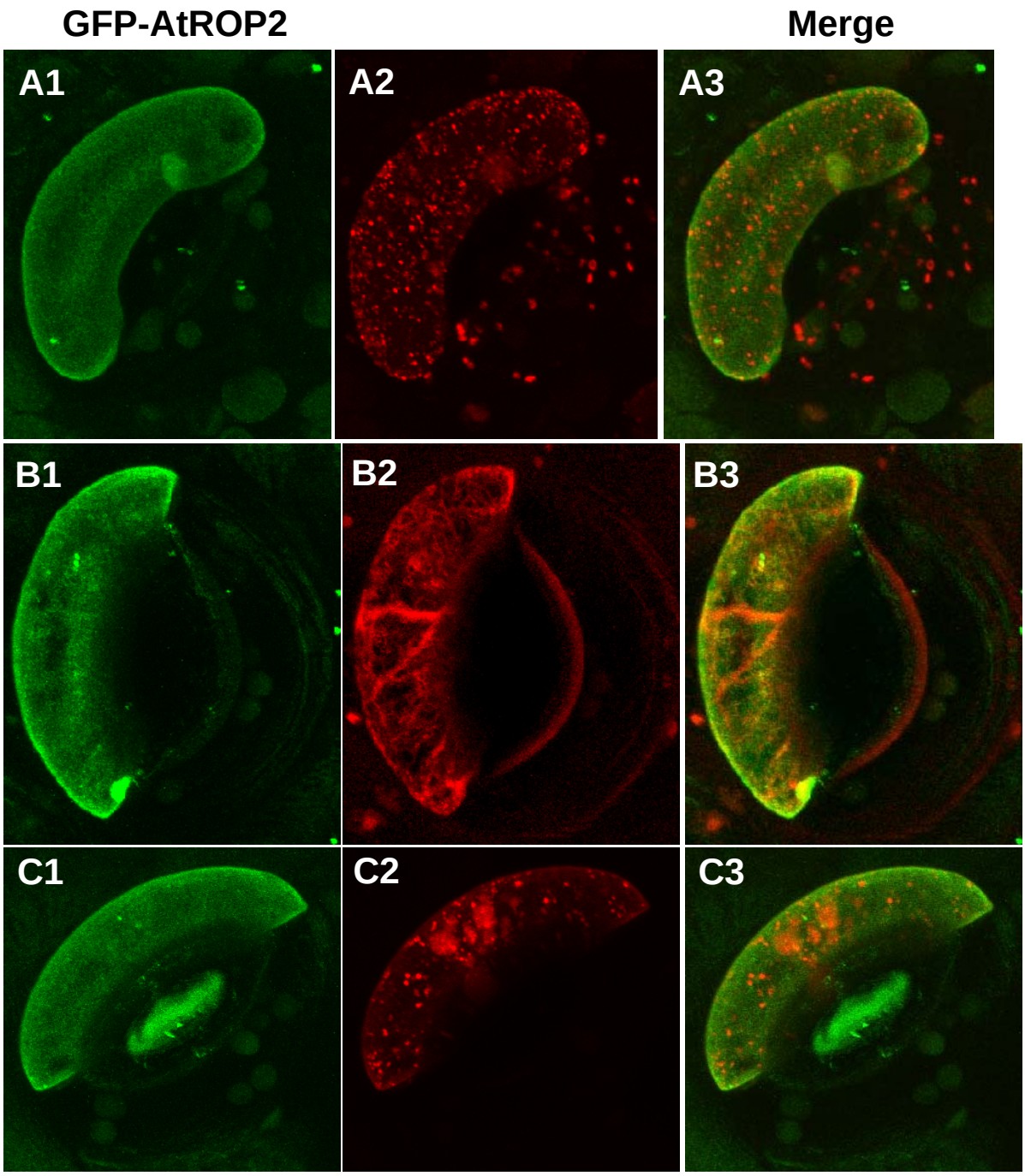


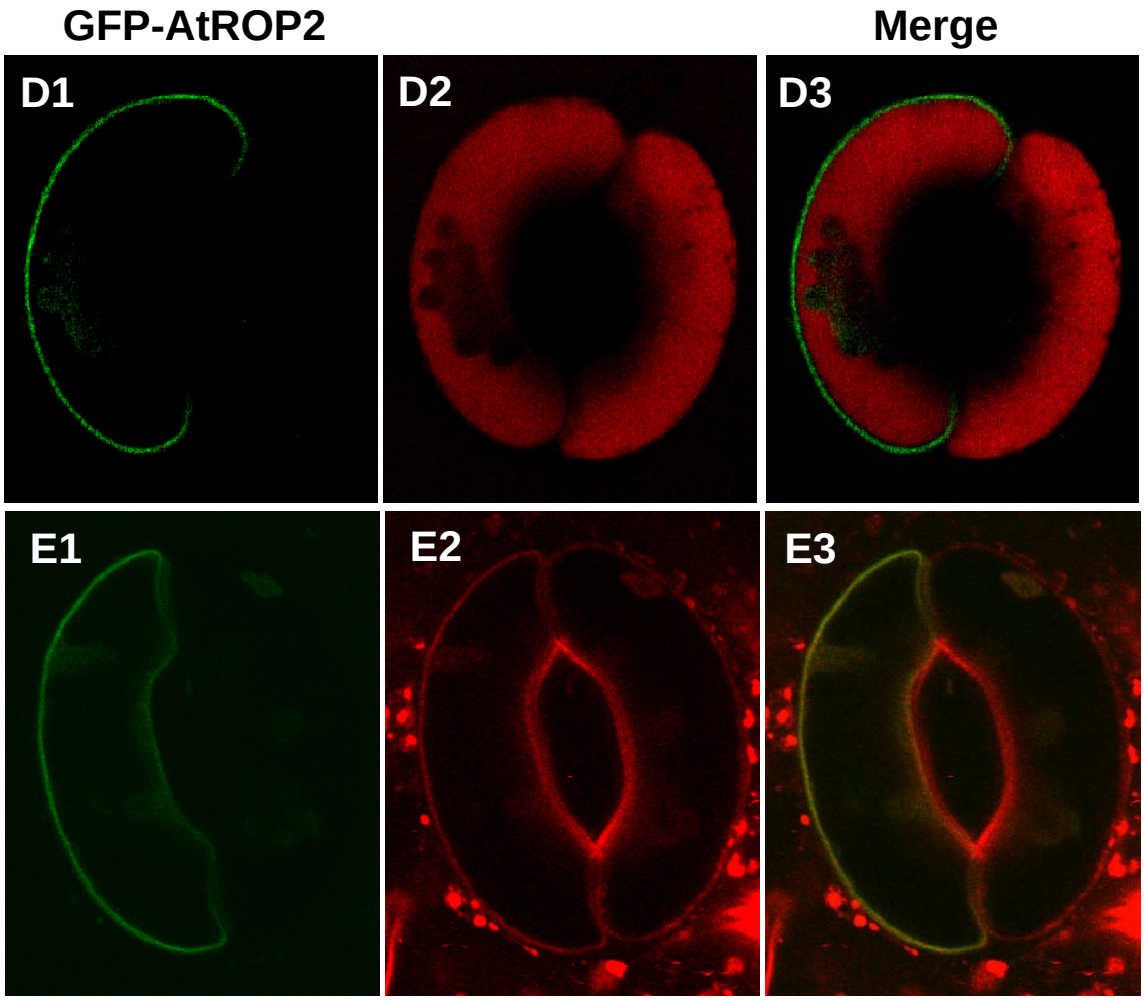
Supplemental Figure 3. The fluorescence intensity of AHA2-GFP (H^+-ATPase, a PM marker, Kim et al; 2001) does not change in guard cells after 2 h of irradiation with white light at intensities of 250 µmol $m^{-2}s^{-1}$.

(A) Fluorescence images of cells obtained before (left) and after (right) 2 h of illumination.

(B) Fluorescence intensity values of AHA2-GFP at the PM and in the cytosol before and after light illumination. See Materials and Methods for assay protocol. Values are averages and S. E. from 21 cells. a.u.; arbitrary unit.

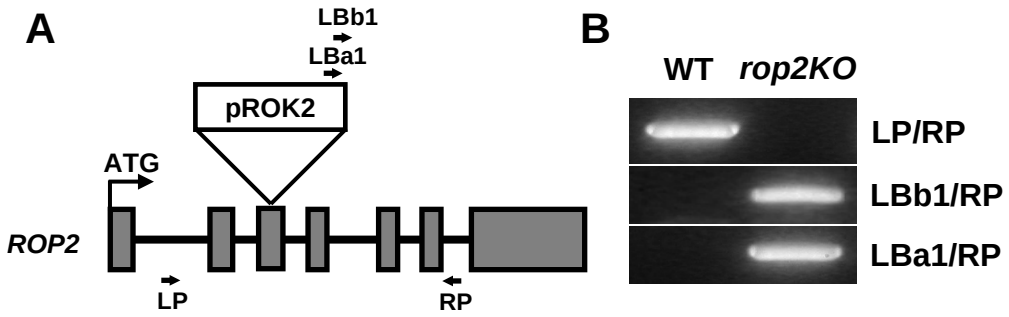
Supplemental Figure 4. GFP-AtROP2 is localized to the PM, and not to the Golgi, ER or vacuole, in guard cells of open stomata under light illumination.





Supplemental Figure 4. GFP-AtROP2 is localized to the PM, and not to the Golgi, ER or vacuole, in guard cells of open stomata under light illumination.
(A, B, C) Intact *V. faba* guard cells co-transformed with *GFP-AtROP2* and a Golgi marker (A2, *ST-RFP*), an ER marker (B2, *BiP-RFP*) or a PVC/vacuolar membrane marker (C2, *RFP-VAM3*). Images are 3 dimensional reconstructions from optical sections obtained using confocal microscopy.
(D, E) Intact *V. faba* guard cells transformed with *GFP-AtROP2* stained with a vacuole marker (D2, monochlorobimane) or a PM marker (E2, FM4-64). To clearly show the boundary, single optical sections from confocal microscopy are presented. Note that in guard cells of open stomata, fluorescence of GFP-AtROP2 was not colocalized with the fluorescence of ST-RFP, BiP-RFP, or RFP-VAM3. Additionally, it did not delineate the monochlorobimane fluorescence. Rather, it was co-localized with the fluorescence of FM4-64.

Supplemental Figure 5. Molecular characterization of the T-DNA insertion in *Arabidopsis ROP2*.



C

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TTTGATCT

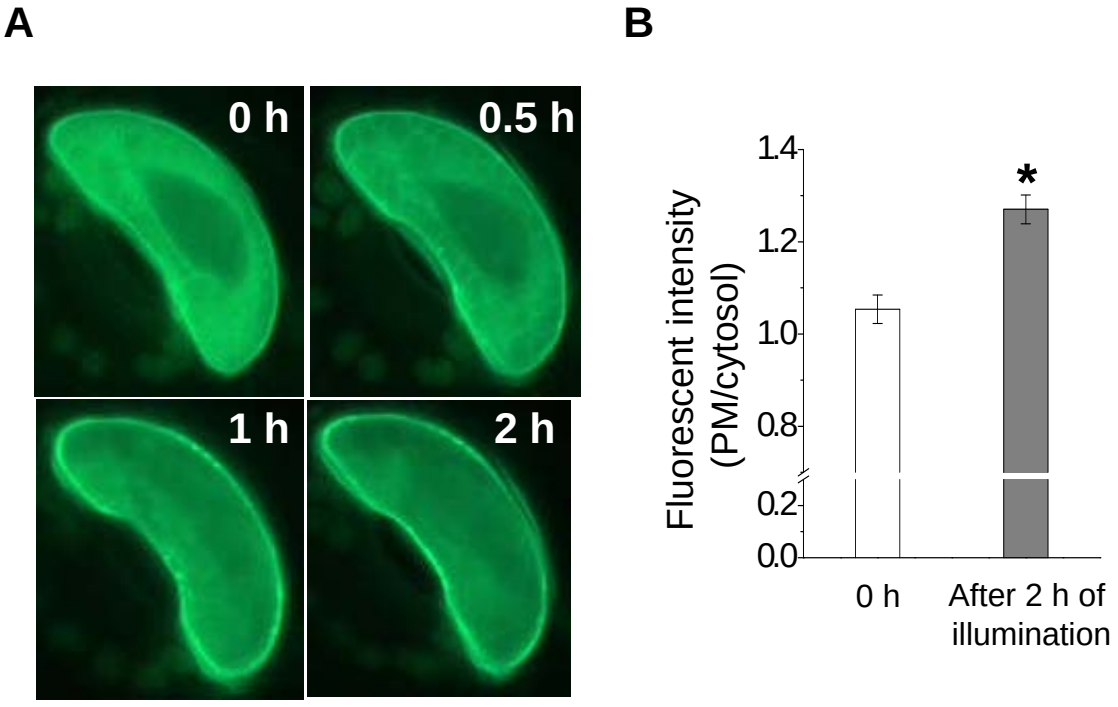
Supplemental Figure 5. Molecular characterization of the T-DNA insertion in *Arabidopsis ROP2*.

(A) Schematic representation of the genomic organization of *ROP2* with the position of the integrated T-DNA in the *rop2-1* plant. Solid bars indicate exons. Solid lines represent introns. Short arrows indicate the direction of primers.

(B) Genotyping of the *rop2-1* plants. Gene-specific primers used for genomic PCR are LP, 5'-TTTTTGCAAATTGGATTCAGGTGTA-3' and RP, 5'-TTCTGCAGAAACACCAAAAGGAAAG-3'. T-DNA–specific primers used are LBa1, 5'-TGGTTCACGTAGTGGGCCATCG-3' and LBb1, 5'-GCGTGGACCGCTTGCTGCAACT-3'.

(C) Nucleotide sequence of the product obtained in PCR using *rop2-1* genomic DNA as template and a primer set LBa1 and RP (*ROP2* gene specific).

Red color letters indicate sequence of T-DNA, in which LBb1 site is marked with >.
Blue color letters indicate sequence of *ROP2* gene.



Supplemental Figure 6. Blue light-induced translocation of GFP-AtROP2 to the PM area.

(A) Intact *V. faba* guard cells expressing *GFP-AtROP2* irradiated with blue light. Leaves bombarded with the *GFP-AtROP2* construct were kept in the dark overnight before being illuminated with blue light at intensities of 20 µmol $m^{-2}s^{-1}$ for the indicated number of hours.

(B) Relative pixel intensity at the PM of GFP-AtROP2 from guard cells transiently transformed with *GFP-AtROP2* before or after 2h of illumination of blue light. The intensity of GFP-AtROP2 localized to the PM (standardized with cytosolic intensity) was measured as described in Figure 3A. The resulting pixel intensity of PM/cytosol is displayed. (mean ± S. E., n=6). * indicates a statistically significant difference ($P < 0.02$).

Supplemental Methods and References

Colocalization of GFP-ROP2

To clarify the plasma membrane localization of ROP2, the localization of ROP2 was compared with that of markers Golgi, ER, prevacuolar compartment (PVC)/vacuolar membrane, tonoplast, or the plasma membrane. The intact *V. faba* leaves were bombarded with GFP-ROP2 alone or mixed with either ST-RFP (Sialyltransferase, a Golgi marker, Kim et al; 2001), BiP-RFP (Chaperon binding protein, an ER marker, Kim et al; 2001) or RFP-VAM3 (a prevacuolar compartment (PVC)/vacuolar membrane marker, Tamura et al; 2007). After overnight incubation under darkness, the leaves were illuminated for 3 h and observed using confocal laser scanning microscopy. To see whether ROP2 delineates vacuole or colocalizes with the plasma membrane, the intact *V. faba* leaves biolistically bombarded with GFP-ROP2 alone was incubated with 10 μM monochlorobimane (Coleman et al; 1997) for 30 min or with 10 μM FM4-64 for 2 min before microscopic observation.

Microscopy

Fluorescence images were obtained using the FV1000 confocal laser scanning

microscope (Olympus, Japan). GFP fluorescence was detected using the 500 to 530 nm spectral settings for emission and 488 nm for excitation. The fluorescence of RFP and FM4-64 were detected using the 555 to 630 nm spectral settings for emission and 543 nm for excitation. The fluorescence of monochlorobimane was detected with 425 to 475 nm spectral settings for emission and 405 nm for excitation.

Supplemental References

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